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GENETIC INTERRELATIONS OF SIX YELLOW-GREEN MUTANTS AND THE MAPPING OF NEGLECTA$_1$ AND YG$_6$ GENES ON CHROMOSOME XI OF TOMATO

IAN A. de la ROCHE

B.Sc. McGill University

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Department of Plant and Soil Science
University of Massachusetts

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INTRODUCTION

With the rediscovery of Mendel's work at the turn of the 20th century, the tomato has played a prominent role in elucidating his laws (28). Subsequent studies in Drosophila by Morgan (27) enabled the assigning of genes to specific loci on chromosomes. The field of investigation, known as chromosome mapping, has advanced to the point that today fine structure analysis is being pursued on the very organism that Morgan originally studied (13).

In higher plants, tomato has become a favorite organism for chromosome mapping, second in importance to corn. To date, over a hundred genetic loci have been determined in eleven of the twelve tomato chromosomes (31).

The aim and purpose of this investigation was:

(1) to determine from dihybrid crosses, the interrelationships among six chlorophyll-deficient mutants of tomato and to isolate and grow to maturity any resulting F_2 double recessive recombinants,

(2) to investigate possible pleiotropism and/or segregation in the yg_6 syndrome,

(3) to confirm the locus of the yg_6 mutant,

(4) to map the neg_1 mutant on chromosome XI.

LITERATURE REVIEW

Since 1954, a series of six "yellow-green" chlorophyll-deficient mutants of tomato have arisen through spontaneous mutation and irradiation of seed (4,6,7,35). These mutants are currently symbolized in the literature as vg_1, vg_2, vg_3, vg_4, vg_5, and vg_6 (32). Chiscon (12) found that vg_2, vg_5, and vg_6 are non-allelic and vg_6 and vg_2 appeared to assort independently from hybrid material. From F_1 tests it was also revealed that vg_3 was non-allelic to vg_2, vg_4 or vg_6, and limited F_2 repulsion data suggested that vg_3 was independently segregating from vg_4 and vg_6. He indicated that vg_3 was linked to vg_2, and exhibited a recombination value of 39.72 ± 4.56 (9). Furthermore, vg_3 gave a 28.72 ± 4.97 recombination value with mottled-2 (md); md is commonly used as a marker for chromosome VI. From this it was postulated that both vg_2 and vg_3 were located on chromosome VI (9).

Whalen analyzed F_2 repulsion data from a double intercross and found 13% recombination between vg_6 and hairless (hl), a marker gene for chromosome XI located at locus 37 (36). A three-point-backcross using markers hl and anthocyaninless$_1$ (a_1) confirmed this result; however, no recombination fraction could be calculated for vg_6 and a_1, because vg_6 was found to be epistatic to a_1.

Whalen (36) compared the theoretical phenotypes expected (without epistasis) and those actually obtained with epistasis (Table 1), to determine the correct order of these three genes.

From the comparison he found that one of the double crossovers in each of the three possible orders occurred as double crossovers only and not again as parentals or single crossovers for that particular order. Furthermore, the double crossover for one possible order appeared as a single crossover in the other two possible orders. Whalen proposed that the correct gene order is the sequence which gives the fewest number of segregates in any of these three classes. It may be noted in Table 1 that the double crossover $++a_1$, of the yg_6 hl a_1 order has the fewest number of segregates. It was therefore concluded that yg_6 hl a_1 was the correct sequence. The position assigned to yg_6 on chromosome XI is indicated in Figure 1.

Table 1. Expected and observed phenotypes for the three possible gene orders (modified Whalen (36)).

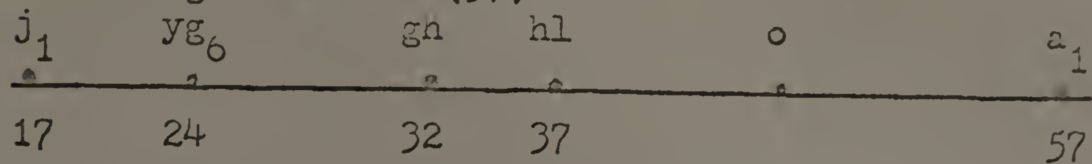
Expected Phenotypic class	yg_6 hl a_1	hl yg_6 a_1	hl a_1 yg_6
Parentals	yg_6 + +[1]	+ yg_6 +[1]	+ + yg_6[2]
	+ hl a_1	hl + a_1	hl a_1 +
Single crossovers region 1	yg_6 hl a_1[2]	+ + a_1	+ a_1 +
	+ + +	hl yg_6 +[2]	hl + yg_6[1]
Single crossovers region 2	yg_6 + a_1[1]	+ yg_6 a_1[1]	+ + +
	+ hl +	hl + +	hl a_1 yg_6[1]
Double crossovers	yg hl +[2]	hl yg_6 a_1[2]	+ a_1 yg_6[2]
	* + + a_1 (15)	* + + + (55)	* hl + + (34)

[1,2] These classes are phenotypically identical within each order because of epistasis.

* This phenotype occurs only as a double crossover in each order.

The number in brackets is the observed number in that class from the three-point backcross.

Figure 1. Diagrammatic section of chromosome XI of Lycopersicon esculentum illustrating the assigned position of yg_6, according to Whalen (37).



o - approximate position of centromere.

Rick (29) reported that mutant $neglecta_1$ (neg_1) has a linkage intensity of 26% with $anthocyaninless_1$ (a_1), a chromosome XI marker found at locus 57.

Whalen (36) observed that the yg_6 mutant comprised a syndrome of three characters: yellow-green first true leaves, elongated hypocotyl, and a greatly reduced amount of anthocyanin development. In analyzing over 10,000 segregates from heterozygote yg_6 plants, no recombination of these three characters with wild types was observed. He suggested that yg_6 may be a case of pleiotropism rather than a complex locus. Chiscon (10) found that the triple recessive mutant, md yg_6 yg_2, was considerably more chlorotic than the single recessive mutants and was lethal in the early seedling stage.

In studying several chlorophyll-deficient mutants of tomato, Whalen found that most of them exhibited a more extreme mutant phenotype when grown at 60 to 70 Farenheit degree temperatures (37).

Certain Xantha and albino mutants are also lethal at the early seedling stage when grown under normal greenhouse conditions (19,20). However, elevated temperatures, high nitrogen content, and high light intensities stimulated chlorophyll synthesis and growth in these mutants (21,22). Lefort successfully grew a lethal Xantha mutant to a more advanced stage by culturing it in a Knopp 0.8% agar medium containing 2% sucrose (25).

METHODS AND MATERIALS

The origin and source of the mutants used in this study are described in Table 2.

Table 2. Origin and source of mutants used in this study.

Gene Symbol	Source	Original Stock
$\underline{yg}_1$	R.W. Robinson	L. esculentum "Rutgers"
$\underline{yg}_2$	A.B. Burdick	L. pimpinellifolium Line 215
$\underline{yg}_3$	R.W. Robinson	L. esculentum "Kokomo"
$\underline{yg}_4$	R.W. Robinson	L. esculentum "Kokomo" m-123
$\underline{yg}_5$	A.B. Burdick	L. esculentum var. cerasiforme
$\underline{yg}_6$	A.B. Burdick	L. esculentum var. cerasiforme
$\underline{neg}_1$	C.M. Rick	L. esculentum "Stubbe's" group 1

$\underline{yg}_1$ (yellow-green$_1$) arose as a spontaneous mutation from a "late fruiting sport of Rutgers" (36). The foliage is yellow-green, especially pronounced in the apical region.

$\underline{yg}_2$ (yellow-green$_2$) was produced by 36 hours of thermal neutron irradiation (6.5×10^8 N^{th} / cm^2 / sec.) of Line 215 seed (4). It is golden-yellow in the apex and turns yellow-green as the leaves mature. the hypocotyl is etiolated and the cotyledons are yellow-green.

$\underline{yg}_3$ (yellow-green$_3$) was induced by 10 hours of thermal neutron treatment of "Kokomo" seed. The leaves are light green with deeply cut and curled edges, giving the plant a ragged appearance (6).

yg_4 (yellow-green$_4$) was produced in cadmium-pretreated, 15,000r x-ray irradiated "Kokomo" seed (4). The seedling has whitish-green foliage and yellow-green cotyledons. Seed set and seed germination is extremely poor.

yg_5 (yellow-green$_5$) was induced through irradiation of seed from line 018 of L. esculentum var. cerasiforme. The mutation is expressed as a golden-yellow seedling with no visible traces of chlorophyll; the cotyledons are pale-yellow. It is the most chlorotic of the mutants in this series. At maturity or under conditions of reduced growth, the foliage turns a very pale green (7).

yg_6 (yellow-green$_6$) resulted from irradiation of L. esculentum var. cerasiforme line 018. The actively growing region is yellow-green with the leaves turning pale green at maturity. The cotyledons are a bright yellow and the hypocotyl is colorless and etiolated under normal greenhouse and field conditions (7).

neg_1 (neglecta$_1$) was one of a group of mutants induced through irradiation of seed by Stubbe (30). The mutant phenotype is expressed in the advanced seedling stage as a mottling on the lower leaves. This develops into a condition of necrotic spotting over the entire surface of the lower leaves, eventually resulting in defoliation. The expression of this gene in the advanced stages is very similar to the symptoms expressed by a severe infestation of red spider mites.

The genetic tester for chromosome XI used in the neg_1 and yg_6 linkage studies was kindly supplied by Dr. L. Butler. It contains the marker genes jointless (j_1), hairless (hl), anthocyaninless (a_1) and fasciated (f_1). The positions of these genes on chromosome XI is illustrated in Figure 1.

The i_1 and hl genes were used as markers in this investigation. Plants homozygous recessive for hairless (hl) are characterized by the complete absence of trichomes except for some glands filled with watery fluid which often give a white speckled appearance. The stems are extremely brittle and are subject to breakage (8). The plants homozygous for the recessive i_1 gene lack an abcission layer in the pedicles of the inflorescence (8).

The relationships of the mutants in the yellow-green series.

The six yellow-green chlorophyll-deficient mutants were cross-pollinated reciprocally in all possible combinations. The resulting F_1 double heterozygotes were studied for extra-chromosomal inheritance and allelism. One or the other and in some cases both of the F_1 plants from a cross were selfed and the F_2 seed was sown in 12 x 15 x 3 inch seedling flats in a sterilized John Innes seeding soil (2 parts soil, 1 part peat moss, 1 part sand, and 60 grams each of superphosphate and ground limestone per bushel of soil mixture). The soil surface of each flat was then treated with diluted Ferradow, a commercial fungicide (1 T. to 8 quarts of water) to prevent growth of molds. All seeds throughout these studies were treated with Arasan, a commercial seed disinfectant and protectant manufactured by DuPont & Co. Four rows, each of twenty-five F_2 seeds were sown per flat, except for the yg_6 x yg_2, yg_5 x yg_2, and yg_6 x yg_5 crosses. In these latter cases approximately 250 F_2 seeds were sown per flat.

A two-point backcross test was also performed with the yg_3 and yg_2 mutants. The yg_3 x yg_2 F_1 dihybrid was crossed with the F_2 yg_3/yg_3 yg_2/yg_2 recombinant. The resulting seeds were sown in the manner described above.

Classification of the F_2 and backcross segregates was made when any of the four expected phenotypes could be distinguished. The procedure was to score and then remove those distinguishable phenotypes at the earliest possible stage to allow space for the other unidentified types to develop. Generally, classification was done in the young seedling stages; however, in some cases it was delayed until maturity. In the latter case, the plants were transplanted into 4 inch clay pots containing the John Innes transplanting media (7 parts soil, 3 parts peat moss, 2 parts sand and 60 grams each of superphosphate and ground limestone per bushel of soil mixture).

Chromosome mapping of yg_6 and neg_1

The yg_6 and neg_1 mutants were crossed to the hl j_1 a_1 f_1 tester. The F_1 generation was selfed and the F_2 seed sown as indicated previously. These plants were transplanted at the advanced seedling stage into 3 inch peat pots and grown in the greenhouse to the first fruit cluster stage, at which time they were transplanted into the field. 1,108 F_2 plants from the yg_6 x tester cross were grown in rows and spaced 5' x 3'. The F_2 plants from neg_1 x tester were seeded in two lots, two months apart. The first lot of 634 plants were planted in the field in June 1965 at 3' x 2' spacing and the second lot of 406 plants were set out in August 1965 at 3' x 3' spacing. The plants from both crosses were scored at approximately the second cluster fruit-set stage when the jointless character could be easily distinguished.

Statistical Methods

The chi-square method was used to test goodness of fit of certain ratios for possible linkage among the members of the yellow-green series and for the chromosome mapping studies with yg_6 and neg_1 (3,14,24,26). The 5% level of significance was employed throughout the studies. Heterogeneity tests were conducted on all individual sets of data from each cross (24). Linkage was determined by the Product Method utilizing Steven's tables (34). The statistical formulae used in this investigation and the reasons for their use are described in Appendix A.

Studies on the yg_6 syndrome

All crosses in which yg_6 was segregating from a heterozygous F_2 plant were observed for possible recombination of the yg_6 syndrome. If recombination was suggested, the plant in which it occurred was selfed and its progeny observed for genetic continuity.

The culture of the lethal double recessive recombinants

The double recessive recombinants from the yg_6 x yg_5, yg_6 x yg_2, and yg_5 x yg_2 crosses succumbed soon after germination when grown under normal greenhouse conditions. When transplanted from the seedling flats into 4" clay pots and grown under a regime of a 75 Farenheit degree temperature and five and one-half hours of supplemental light, the plants grew reasonably well, allowing for phenotypic comparisons with close relatives and other members of the yg series.

RESULTS

Interrelationship among the mutants of the yellow-green series

When the six yellow-green mutants were crossed reciprocally in all possible combinations, the resulting F_1's were all phenotypically of the wild type. This suggested that the mutants are genetically inherited, are non-allelic, and no indication of cytoplasmic inheritance was suggested.

The observed F_2 phenotypes for each of the fifteen yellow-green series of intercrosses, in Table 3, represent the family totals for individual lots, homogeneous for chi-square A and B single factor segregation and for linkage. The results of these heterogeneity tests are recorded in the Appendix B.

Table 3. Observed F_2 phenotypes for each of the fifteen yellow-green series of double intercrosses in repulsion.

A^* x B	F_2 Phenotypes + +	+ b	a +	a b	Total	Total Seeded
$\underline{yg}_1$ x $\underline{yg}_2$	208	92	88	25	473	500
$\underline{yg}_3$ x $\underline{yg}_1$	281	74	85	16	456	500
$\underline{yg}_1$ x $\underline{yg}_4$	335	55	107	12	509	600
$\underline{yg}_5$ x $\underline{yg}_1$	295	97	86	19	497	600
$\underline{yg}_6$ x $\underline{yg}_1$	405	142	146	36	729	800
$\underline{yg}_3$ x $\underline{yg}_2$	367	110	78	27	582	600
$\underline{yg}_4$ x $\underline{yg}_2$	576	205	95	18	894	1000
$\underline{yg}_5$ x $\underline{yg}_2$	596	202	161	55	1014	
$\underline{yg}_6$ x $\underline{yg}_2$	645	214	214	63	1136	
$\underline{yg}_4$ x $\underline{yg}_3$	359	98	28	0	485	1000
$\underline{yg}_5$ x $\underline{yg}_3$	362	88	87	21	558	600
$\underline{yg}_6$ x $\underline{yg}_3$	423	129	89	20	661	700
$\underline{yg}_4$ x $\underline{yg}_5$	946	305	12	1	1264	1501
$\underline{yg}_4$ x $\underline{yg}_6$	511	72	26	4	613	700
$\underline{yg}_6$ x $\underline{yg}_5$	453	176	143	38	810	810

* A represents the maternal parent.

Table 4, shows the three correct chi-square parameters on the F_2 total for each cross.

Table 4. Analysis of the dihybrid crosses from all combinations of the yellow-green series of mutants.

A x B	X^2_A	X^2_B	X^2_L	Recombination Value
yg_1 x yg_2	0.13	0.02	0.52	50
yg_3 x yg_1	1.98	6.74**	0	50
yg_1 x yg_4	0.71	38.04**	1.29	50
yg_5 x yg_1	3.98*	0.42	2.05	50
yg_6 x yg_1 [a]	0	0.13	2.78	50
yg_3 x yg_2	15.03**	0.66	0.34	50
yg_4 x yg_2	72.84**	0	5.61	41.15 ± 4.07
yg_5 x yg_2	7.40**	0.06	0	50
yg_6 x yg_2	0.23	0.23	0.56	50
yg_4 x yg_3 [a]	94.60**	5.69*	6.25*	50
yg_5 x yg_3 [a]	9.48**	8.89**	0	50
yg_6 x yg_3 [a]	25.53**	2.13	1.31	50
yg_4 x yg_5	386.10**	0.38	1.15	50
yg_4 x yg_6 [a]	131.09**	51.25**	0.02	50
yg_6 x yg_5	3.04	0.87	3.60	50

* indicates a 5% level of significance.
** indicates a 1% level of significance.
a indicates reciprocal crosses.

In the crosses involving $\underline{vg}_1$, the F_2 segregates were transplanted from the seed flat because of the inability to distinguish, at the seedling stage, between $\underline{vg}_1$ parental types and wild type recombinants as well as between the recessive recombinant and the other parental type. This difficulty was overcome by supplying the transplants with a high level of nutrition and supplemental light. The F_2 segregates grew rapidly, accentuating the phenotypic differences among the four F_2 segregates. Unfortunately, this was not completely successful with the F_2 segregates from the $\underline{vg}_1$ x $\underline{vg}_4$ cross, since some of the $\underline{vg}_1$ F_2 parental types were misclassified as F_2 wild type recombinants. This can be seen from the data in Table 4, where a significant chi-square for $\underline{vg}_1$ segregation was obtained. However, the correction was made when the linkage chi-square was calculated.

The F_2 segregates from the other ten crosses shown in Table 3 were easily distinguished in the advanced seedling stage and they were scored directly from the seed flats.

Non-significant chi-square values for linkage were obtained for all the combinations tested except for the $\underline{vg}_4$ x $\underline{vg}_2$ and $\underline{vg}_4$ x $\underline{vg}_3$ crosses (Table 4). Originally 532 F_2 segregates were analyzed from the $\underline{vg}_4$ x $\underline{vg}_2$ cross, giving a contingency chi-square for linkage of 4.61 and a recombination value of 39.48 $\pm$ 8.65. By increasing the F_2 population to 894, the contingency chi-square for linkage increased to 5.61 and the recombination value changed to 41.15 $\pm$ 4.07 (Table 4). This drop in standard error by one-half, further strengthened the possibility of linkage. From 485 segregates of the $\underline{vg}_4$ x $\underline{vg}_3$ cross, no $\underline{vg}_4/\underline{vg}_4$ $\underline{vg}/\underline{vg}_3$ recombinants were isolated.

The yg_3 and yg_2 mutants were also tested in a double backcross (Tables 5 and 6). The results confirmed the independence of yg_3 and yg_2.

Table 5. Observed phenotypes from the yg_3 and yg_2 double backcross.

A x B	+ +	+ yg_2	yg_3 +	$yg_2 yg_2$	Total	Total Seeded
yg_3 x yg_2	29	30	32	25	116	130

Table 6. Analysis of yg_3 x yg_2 double backcross data.

A x B	X^2A	X^2B	X^2L	Recombination Value
yg_3 x yg_2	0.03	0.31	0.55	0.50

A total of 3179 F_2 segregates were classified from the original yg_6 x yg_5 population. The data were grouped according to levels of germination rather than tested for heterogeneity on a per flat basis. A significant chi-square value of 5.99 was obtained for segregation of yg_6 in the total population. However, when the analysis was repeated on the F_2 data, which exhibited 100 per cent germination, single factor segregation of yg_6 was not significantly disturbed. Early in the investigation, it was felt that yg_6 segregates were initially scored before all the yg_6 parental types had germinated. Later, classification was delayed until the late seedling stage.

Generally, the F_2 parental phenotypes appeared to be phenotypically identical to the parents irrespective of the cross they were from. One exception was noted in the interspecific crosses involving yg_2 from

L. pimpinellifolium and the other five yellow-green mutants from *L. esculentum*. Here, the leaf size and shape of the yg_2 F_2 parental type generally was expressed as an intermediate between the two species. In all cases, the F_2 wild type recombinant resembled the F_1 parent.

Description of the recessive recombinants

In all the crosses involving yg_1, the recessive recombinants resembled the other parent for all characters except chlorophyll content. The yg_2/yg_2 yg_3/yg_3 resembled neither parent completely. From the 52 recessive recombinant types examined, segregation of the yg_2 character, etiolated hypocotyl, seems to be occurring. The leaflets of the seedlings were marginally curled upwards to a much greated extent than with the yg_3/yg_3 +/+ parent. However, the amount of anthocyanin in the hypocotyl closely resembled the level expressed by the yg_2/yg_2 +/+ parent.

The yg_2/yg_2 yg_4/yg_4 was lethal in the seedling stage. The cotyledons appeared as albinos with only a trace of anthocyanin in the hypocotyl. All of the eighteen recessive recombinants studied had etiolated hypocotyls. The leaflets were completely white and the seedlings died about one week after germination.

The cotyledons and hypocotyl of the yg_2/yg_2 yg_5/yg_5 were very similar to those of the yg_2/yg_2 +/+ parent. Under normal greenhouse conditions, the developing leaflets were white with faint traces of yellow color. These mutant combinations were lethal in the early seedling stage.

The $\underline{yg}_2/\underline{yg}_2$ $\underline{yg}_6/\underline{yg}_6$ F_2 segregates have pale yellow-green cotyledons, a colorless elongated hypocotyl and pale yellow leaflets with patches of white. This recessive recombinant was lethal at the mid-seedling stage. The $\underline{yg}_3/\underline{yg}_3$ $\underline{yg}_5/\underline{yg}_5$ had yellow-white foliage and a ragged appearance characteristic of the $\underline{yg}_3$.

Chiscon's description of $\underline{yg}_3/\underline{yg}_3$ $\underline{yg}_6/\underline{yg}_6$ agreed well with the twenty recessive recombinant segregates which appeared from the $\underline{yg}_3$ x $\underline{yg}_6$ cross (10). All the mutants had colorless elongated hypocotyls with deeply cut and curled, yellow-green leaflets.

Only one recessive recombinant appeared in the F_2 population from the $\underline{yg}_4$ x $\underline{yg}_5$ cross. The cotyledons were albinotic and closely resembled the $\underline{yg}_4$ in shape. It died soon after germination and before the epicotyl fully developed.

The $\underline{yg}_4/\underline{yg}_4$ $\underline{yg}_6/\underline{yg}_6$ died before the first true leaves appeared. It had a colorless elongated hypocotyl and albinotic cotyledons.

The cotyledons of the $\underline{yg}_5/\underline{yg}_5$ $\underline{yg}_6/\underline{yg}_6$ F_2 segregate were a lighter bleached color than either of the parents. It had the elongated hypocotyl characteristics of the $\underline{yg}_6$ and its leaflets were a pale yellow-green with large patches of white. This combination proved to be lethal at the young seedling stage. In all cases, the recessive recombinant was more chlorophyll-deficient than either of the parents.

The F_2 recessive recombinants from the $\underline{yg}_5$ x $\underline{yg}_2$, $\underline{yg}_2$ x $\underline{yg}_6$, and $\underline{yg}_2$ x $\underline{yg}_5$ crosses which were grown at 70° F. and 16 hours of light, all grew to a more advanced stage.

The $\underline{yg}_2/\underline{yg}_2$ $\underline{yg}_5/\underline{yg}_5$ continued to develop until maturity; however, recessive recombinants from $\underline{yg}_5$ x $\underline{yg}_2$ and $\underline{yg}_2$ x $\underline{yg}_6$ crosses died at the advanced seedling stage.

It is apparent from the data in Table 4 that the vg_4 gene exhibits a very highly significant disturbed segregation within crosses where it participated. This is especially noticeable in the vg_4 x vg_5 cross where a 386.10 chi-square value was obtained.

The crosses designated by "a" in Table 4 were performed reciprocally. Four of these crosses, vg_4 x vg_3, vg_5 x vg_3, vg_6 x vg_3 and vg_6 x vg_4 are particularly noteworthy because one or both of the single factors exhibit disturbed segregation. A heterogeneity test performed on the reciprocal crosses of these data revealed that the direction of the cross had no effect on single factor segregation or linkage.

Pleiotropism in the vg_6 syndrome

When the data from all crosses, segregating for vg_6 from the heterozygotes were grouped, a total of 11,284 plants had been observed for possible recombination within the vg_6 syndrome. In several instances it was found that vg_6 segregates had accumulated a small amount of anthocyanin in the hypocotyl but none of these approached the wild type in intensity of the pigment. Several vg_6 types containing anthocyanin were selfed but the resulting progenies all exhibited colorless hypocotyls.

Chromosome mapping of yg_6

In the yg_6 mapping studies, only the hl and i_1 marker genes were used from the multiple tester. The total F_2 repulsion data were regrouped according to separate yg_6 x hl and yg_6 x i_1 dihybrid crosses (Table 7). The analysis of these data indicated that yg_6 was linked to both hl and i_1, with recombination values of 16.43 ± 4.51 and 29.06 ± 5.09 respectively (Table 8). Reconciling these values with the known positions of hl and i_1 on chromosome XI, the position of yg_6 would fall between hl and a_1, as shown in Figure 2a. Assuming 13 crossover units from hl (37), yg_6 would be located 33 units from i_1 and 7 units from a_1 (Figure 2b).

Table 7. F_2 repulsion data from yg_6 x tester cross.

A x B	F_2 Phenotypes				
	+ +	+ b	a +	a b	Total
yg_6 x hl	632	207	264	5	1108
yg_6 x i_1	635	204	252	17	1108

Table 8. Analysis of F_2 repulsion data from yg_6 x tester cross.

A x B	X^2A	X^2B	X^2L	Recombination Value
yg_6 x hl	0.27	20.03**	70.00**	16.43 ± 4.51
yg_6 x i_1	0.31	15.10**	41.31**	29.06 ± 5.09

Figure 2a. Approximate location of yg_6 on chromosome XI.

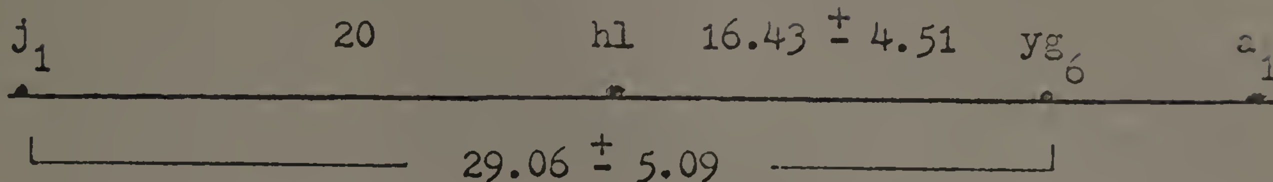


Figure 2b. Suggested position of yg_6 on chromosome XI.

Chromosome mapping of neglecta$_1$

A heterogeneity test on the two lots of neg_1 x multiple tester F_2 repulsion data revealed that they were homogeneous for the three parameters under consideration (Table XX, XXI Appendix). The data in Table 9 show the number of individuals in the four segregation classes, when the total data from the two lots were regrouped as neg_1 x hl and neg_1 x a_1 dihybrid crosses. Again, as with the yg_6 mapping studies, only the hl and j_1 genes were considered from the multiple tester.

Rick's data from his neg_1 and a_1 linkage studies (30) were included in Table 9 as it is pertinent for the positioning of neg_1. His data were reworked according to the method outlined in the Methods and Materials. A 24.21 ± 4.41 recombination value was obtained as shown in Table 10 along with the determined recombination values for neg_1 and hl and neg_1 and j_1. If these recombination values are mapped relative to the known positions of j_1, hl, and a_1, neg_1 would be positioned between j_1 and hl (Figure 3a). Further,

positions 28.65 to 30.28 inclusive is the range common to the three recombination values. Consequently, $\underline{neg}_1$ is positioned at approximately locus 29 on chromosome XI (Figure 3b).

Table 9. F_2 repulsion data from $\underline{neg}_1$ x $\underline{tester}$ cross.

A x B	+ +	+ b	a +	a b	Total
$\underline{neg}_1$ x $\underline{hl}$	649	279	263	3	1194
$\underline{neg}_1$ x $\underline{j}_1$	672	256	264	2	1194
$\underline{neg}_1$ x $\underline{a}_1$	590	242	197	11	1040

Table 10. Analysis of F_2 repulsion data from $\underline{neg}_1$ x $\underline{tester}$ cross.

A x B	X^2A	X^2B	X^2L	Recombination Value
$\underline{neg}_1$ x $\underline{hl}$	4.57*	1.14	97.57**	11.33 ± 4.04
$\underline{neg}_1$ x $\underline{j}_1$	4.57*	7.15**	89.48**	9.85 ± 3.43
$\underline{neg}_1$ x $\underline{a}_1$	13.87**	0.25	51.19**	24.21 ± 4.14

Figure 3a. Approximate location of $\underline{neg}_1$ on chromosome XI.

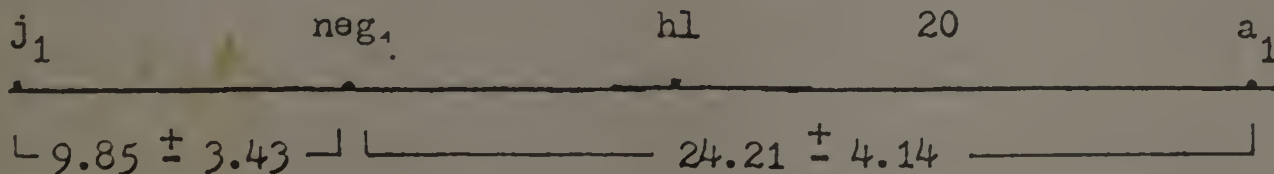


Figure 3b. Suggested positions of $\underline{neg}_1$ on chromosome XI.

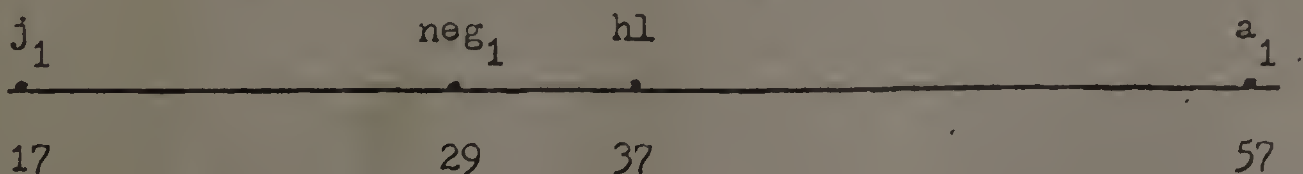


Table 11 records the amount of information obtained relative to the testcross for all the F_2 repulsion crosses performed in this study.

Table 11. Efficiency of F_2 repulsion data relative to the double back-cross.

A x B	Total F_2 Segregates	Recombination Value	% Efficiency	Effective No.
yg_1 x yg_2	473	0.50	44.44	210
yg_3 x yg_1	456	0.50	44.44	203
yg_1 x yg_4	509	0.50	44.44	226
yg_5 x yg_1	497	0.50	44.44	221
yg_6 x yg_1	729	0.50	44.44	324
yg_3 x yg_2	582	0.50	44.44	259
yg_4 x yg_2	894	0.37*	32.20	288
yg_5 x yg_2	1014	0.50	44.44	451
yg_6 x yg_2	1136	0.50	44.44	505
yg_4 x yg_3	485	0.50	44.44	216
yg_5 x yg_3	558	0.50	44.44	248
yg_6 x yg_3	661	0.50	44.44	294
yg_4 x yg_5	1264	0.50	44.44	562
yg_4 x yg_6	613	0.50	44.44	272
yg_6 x yg_5	810	0.50	44.44	360
yg_6 x hl	1108	0.13	11.79	131
yg_6 x i_1	1108	0.33	28.66	318
neg_1 x hl	1194	0.08	7.47	89
neg_1 x hl	1194	0.12	10.94	131
neg_1 x a_1	1040	0.28	24.35	253

* The lower range of the recombination fraction was used to give the minimum possible efficiency.

DISCUSSION

The results from reciprocal crosses demonstrate that the inheritance of the yellow-green series of chlorophyll-deficient mutants is nuclear and not under the influence of extra-chromosomal control. Chiscon's observations that yg_2, yg_5, and yg_6 are non-allelic and that yg_3 was non-allelic to yg_2, yg_4, and yg_6 were confirmed (9,12). Also, the F_1's from the other nine possible combinations expressed themselves as "wild type". It was concluded therefore that all six of the yellow-green mutants in the series were located at separate genetic loci.

The F_2 repulsion data from the yg_3 x yg_2 cross, suggested that these two genes were segregating independently. This was later confirmed by a double backcross (Table 6). These findings are in conflict with the results of Chiscon (9) who reported that yg_2 is linked to yg_3 with a recombination value of 39.72 ± 4.56. An analysis of his raw data (9), however, reveals an adjusted contingency chi-square for linkage of 2.45 which is not significant at the 5% level. It is general procedure to use the chi-square to first detect linkage, and if present, then to determine the recombination fraction (3). The possibility of having a recombination value of approximately 40% with a non-significant chi-square for linkage becomes apparent when we consider that the recombination between two distant genes is usually an under estimate of the map distance (16). Therefore, it seems that yg_2 and yg_3 are indeed independent of one another.

Also, since yg_2 was assigned to chromosome XI because of apparent linkage to yg_3, further consideration must be given to this assignment in light of the above conclusion.

The failure to observe any yg_4/yg_4 yg_3/yg_3 segregates from the $yg_4 \times yg_3$ cross is consistent with the observations made by Chiscon (9). It is possible that a recessive recombinant was not observed because it was indistinguishable from the yg_4 parental type. This seems plausible because yg_4 is the more chlorophyll-deficient of the two parents. These two genes exhibited a significant chi-square for linkage mainly because of the absence of the recessive recombinant. A recombination value could not be determined for the same reason.

Several of the F_2 parental types were selfed, in hopes of obtaining a recessive recombinant through segregation but these attempts were unsuccessful.

It seems likely that these mutants are independent since definite linkage has been obtained between yg_3, (a gene marker for chromosome VI (9)) and yg_4 has been shown to be loosely linked to anthocyaninless loser (al) which is on chromosome VIII (5).

F_2 data from the $yg_4 \times yg_2$ intercross consistently indicated loose linkage between these two genes (Table 4). If this were the case, it would not be inconsistent with the above results. As suggested previously, however, a high recombination fraction is not a very accurate determination of map distance. Even though linkage is questionable in this case, it may merit further analysis with a marker gene from chromosome VIII.

It was demonstrated that *yg*$_3$ was not linked to *yg*$_6$ which is consistent with the results obtained by Chiscon (9). The results also indicated the other eleven possible relationships among the six yellow-green mutants were independent. The fourteen recessive recombinants studied from intercrosses in the yellow-green series were more chlorophyll-deficient in all cases than either of the parents. This agrees with Chiscon's results from chlorophyll determinations on four of the recessive recombinants (11).

Several of these recessive recombinant combinations were lethal in the seedling stage under normal greenhouse conditions. Using elevated temperature and supplemental light, it was possible to grow the yg_2/yg_2 yg_5/yg_5 recombinant to maturity and to grow the yg_6/yg_6 yg_5/yg_5 and yg_6/yg_6 yg_2/yg_2 recombinants to an advanced seedling stage.

Seed germination of the *yg*$_4$ mutant is always extremely poor. It was thought initially that differential viability of *yg*$_4$ F_2 parental type seed could explain the highly significant, disturbed segregation of *yg*$_4$ in combination with the five other yellow-green mutants. However, a critical examination of the *yg*$_4$ x *yg*$_5$ F_2 data revealed that this explanation alone was unsatisfactory. A significant chi-square value of 66.19 was obtained for *yg*$_4$ segregation when the portion of the F_2 population that failed to germinate was assumed to be either yg_4/yg_4 +/- and/or yg_4/yg_4 yg_5/yg_5 plants. Apparently, the F_2 produced a deficiency of yg_4/yg_4 +/- and yg_4/yg_4 yg_5/yg_5 seed, probably due to some irregularity occurring as late as embryonic development. The aberrant response is consistent with the observations made by Whalen (37), who reported that seed set in dihybrid crosses on *yg*$_4$ was extremely low.

This same phenomenon may be occurring in the yg_4 x yg_6 cross as a heterogeneity test indicates the F_2 segregations from this and the yg_4 x yg_5 cross are behaving homogeneously with respect to yg_4 segregation. If this is the case, the direction in which the cross is made appears to have no effect, i.e., F_2 data from yg_4 x yg_6 reciprocal crosses are homogeneous.

Studies are presently under way to determine if the yg_4 segregation of the reciprocal yg_4 x yg_5 cross is affected in the same manner. Although the other three crosses, in which yg_4 participated, exhibit a highly significant disturbed segregation, the degree of disturbance was not homogeneous with yg_4 x yg_5 and yg_4 x yg_6 crosses.

It was also noted that yg_4 is from a stock of L. esculentum while yg_5 and yg_6 are from L. esculentum var. cerasiforme. No cases of differential gametic or zygotic lethality, however, have been reported as a result of the above intervarietal cross (37).

Limited pollen viability tests using tetrazolium red (33), suggest that yg_4/yg_4 +/+ plants exhibit a high degree of pollen abortion as compared to +/+ yg_5/yg_5. In view of the above, the same phenomenon might be expected for the female gamete.

The fact that yg_5 segregation is not affected in the F_2, may suggest that the irregularity is due to the yg_4 gene (pleiotropism) or the presence of closely linked genes. It has been observed that those yg_4/yg_4 +/- F_2 plants that do develop generally appear quite distorted. This distortion fails to manifest itself when homozygous lines of yg_4 are selfed. This suggests a gametic incompatibility at fertilization or perhaps even later in zygotic development.

The collapse of zygotes during embryonic stages is often due to disturbances in embryo-endosperm relationships and has been alluded to as somatoplastic sterility by Allard (1).

When all the $\underline{yg}_6$ segregates from this experiment are combined with the 10,000 observed by Whalen (36), a total of more than 21,000 from $\underline{yg}_6/\underline{yg}_6$ +/+ heterozygotes, it is especially noteworthy that recombinations of characters within the syndrome had not occurred.

Though, it seems that pleiotropism may be the case in the $\underline{yg}_6$ syndrome, closely linked loci can not be disregarded until many more individuals are studied. An experiment is presently under way which will result in the examination of more than 1000,000 segregates.

As previously mentioned, the data from the $\underline{yg}_6$ x $\underline{yg}_5$ double intercross indicated that $\underline{yg}_6$ may be slower in germinating than either the wild type recombinants and/or the $\underline{yg}_5$ F_2 parental types. A series of germination studies were conducted using the six yellow-green mutants and it appeared that $\underline{yg}_6$ germination was delayed by approximately three days when compared with the five other mutants. Examination of $\underline{yg}$ seedlings grown in tissue culture suggested poor rootlet initiation when compared to the extremely-deficient $\underline{yg}_5$. It may well be that late root initiation or restricted early development of some sort is yet another characteristic included within the $\underline{yg}_6$ syndrome.

A closer look at the three possible gene orders in Table 1 reveals that the last column, $\underline{hl}$ $\underline{i}_1$ $\underline{yg}_6$ order, can be disregarded because the linear distance from $\underline{hl}$ to $\underline{yg}_6$ can not be greater than the distance between $\underline{hl}$ and $\underline{a}_1$.

It is clear that the selection of either vg_6 hl a_1 or hl vg_6 a_1 orders depends entirely on the classification of $++a_1$ and $+++$ double crossover classes. In previous studies with a_1, we noted that its phenotypic expression is under great influence from the environment and under certain conditions it is practically indistinguishable from wild type, hence discouraging its use in linkage studies.

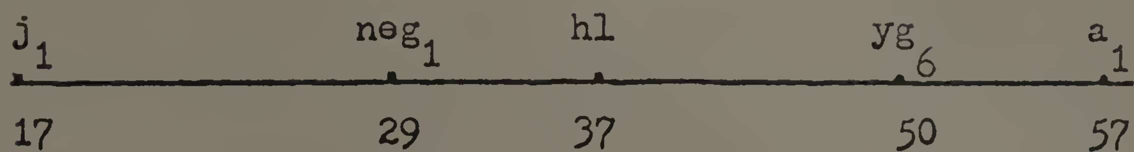
A goodness-of-fit test on Whalen's data using the recombination fractions of 33% and 7% as the distance between vg_6 and a_1 for the vg_6 hl a_1 and hl vg_6 a_1 orders respectively, resulted in a significant chi-square in both cases.

It is seen from Table 8 that 16.43 ± 4.51 falls within the 13% crossover range for vg_6 and hl, confirming Whalen's initial results (37). However, the 29.06 ± 5.09% recombination between vg_6 and i_1 agree with the 33 crossover units rather than the 7 crossover units that would be expected according to Whalen's suggested position. The F_2 repulsion data from the $vg_6 \times i_1$ cross is as efficient as studying 318 segregates from a double backcross (Table 11).

Therefore, it is suggested that vg_6, instead of being at position 24 on chromosome XI (37), is really located at position 50, between hl and a_1, (Figure 4). A confirmatory three-point backcross test with hl and i_1 is presently in progress.

As suggested in the results, neg_1 is positioned between i_1 and hl at approximately locus 29 on chromosome XI (Figure 4). A homozygous triple recessive, i_1 neg_1 hl, is currently being synthesized for the confirmatory three-point backcross.

Figure 4. Suggested positions of neg_1 and yg_6 on chromosome XI.



SUMMARY

Studies were conducted to determine the genetic interrelations of six yellow-green mutants and to map the neglecta$_1$ and yg$_6$ genes on chromosome XI of tomato.

(1) The inheritance of yg$_1$, yg$_2$, yg$_3$, yg$_4$, yg$_5$, and yg$_6$ chlorophyll-deficient mutants was found to be genetically controlled and non-allelic.

(2) F_2 repulsion data from double intercrosses of all these mutants indicated that they assorted independently, except for possibly yg$_2$ and yg$_4$.

(3) In all cases, the double recombinant mutants appeared to be more chlorophyll-deficient than either of their parents.

(4) The same phenomenon of disturbed segregation of the yg$_4$ mutant was considered, as well as the level at which it may be occurring.

(5) Evidence was presented which suggests that yg$_6$ is located at position 50 rather than 24 on chromosome XI.

(6) Neglecta$_1$ appears to be located at approximately locus 29 on chromosome XI.

(7) Since no recombination was observed in the characters of the yg$_6$ syndrome, it must still be considered a case of pleiotropism rather than independent effects of a complex locus.

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APPENDIX A

Statistical Methods

When F_2 repulsion data was being analyzed, the goodness of fit chi-square was portioned into chi-square for single factor segregation of A and B and chi-square for linkage between A and B, each with 1 degree of freedom. The formulae used are shown below (3).

$$X^2 \text{ A factor segregation} = \frac{(a + b - 3c - 3d)^2}{3n}$$

$$X^2 \text{ B factor segregation} = \frac{(a - 3b + c - 3d)^2}{3n}$$

$$X^2 \text{ linkage between A and B} = \frac{(a - 3b - 3c + 9d)^2}{9n}$$

Where _n_ represents the total number of F_2 segregates and _a_, _b_, _c_, and _d_ the observed number of F_2 segregates for each of the four expected phenotypes. Whenever the number of any class was less than 10, a correction for continuity was used (24). This was accomplished by reducing the quantity inside the brackets by two in absolute magnitude (14). If either factor A or B, or both, exhibited a disturbed segregation, the chi-square for linkage formula indicated above is not accurate and instead a contingency chi-square test is employed (26).

$$\text{Contingency } X^2 \text{ for linkage between A and B} = \frac{(ad-bc)^2\, n}{(a+b)(c+d)(a+c)(b+d)}$$

When the need for a continuity correction is indicated in the contingency chi-square, the following short cut formula suggested by Leclerq, _et._ _al._ (24) was used.

$$\text{Contingency } X^2 \text{ for linkage} = \frac{(ad-bc-n/2)^2\, n}{(a+b)(c+d)(a+c)(b+d)}$$

The following formula was used when a single factor disturbance was due to a recessive being classified as a dominant type (3).

$$X^2 \text{ linkage between A and B} = \frac{16(ad-bc)^2}{3n(a+b)(c+d)}$$

In the analysis of double backcross data, the following formulae were employed (3).

$$X^2 \text{ A factor segregation} = \frac{(a+b-c-d)^2}{n}$$

$$X^2 \text{ B factor segregation} = \frac{(a-b+c-d)^2}{n}$$

$$X^2 \text{ linkage between A and B} = \frac{(a-b-c+d)^2}{n}$$

Tests for Heterogeneity

The additive property of the chi-square enables the testing for homogeneity among separate lots of F_2 segregates from a particular F_1 genotype (24). The F_2 repulsion data from the yellow-green series of crosses were first tested on a per flat basis for factor A and B segregation and possible linkage. The data from the homogeneous lots were grouped and the analysis repeated on the totals (3).

Since Yates correction for continuity is non-additive (14), it was not used when testing for heterogeneity. It was used, however, in the analysis performed on the total from homogeneous lots. In crosses that exhibited single factor segregation, and where F_2 data had also been gathered from reciprocal crosses, a test for heterogeneity was performed to determine if these disturbances could be attributed to non-chromosomal effects.

Determination of linkage intensities

When single factor segregation is good, the product method is preferred over the maximum likelihood for the determination of linkage because of the ease of computation when suitable tables are available (34). Several tables are available for the determination of linkage by the product method, Immer (23), Fisher and Balmukand (18), and Stevens (34). However Fisher and Balmukand's and Immer's tables have the disadvantage of involving interpolation whereas in Steven's tables, the recombination fraction and standard error can be obtained directly and the interval between values is much smaller. Bailey (2) indicates that when one factor exhibits disturbed segregation, there is a loss of efficiency in the product method. Mather (26) feels that this efficiency loss is only slight and advocates the product method over the maximum likelihood method, especially when the cross is in repulsion. In this case the recombination fraction from the table remains unchanged but the standard error must be corrected (34). Fisher (18) has derived the following variance formula for crosses that exhibit disturbed segregation.

$$V_\theta = \frac{\theta(1-\theta)(2+\theta)}{(1+2\theta)^2} \frac{2(a+b) + \theta(a+b+9c+9d)}{4(a+b)\ (c+d)}$$

Where θ is the recombination fraction squared ($\bar{y}^2$) for repulsion data and $\theta = (1-\bar{y})^2$ for coupling data. From this variance, the standard error of the recombination can be determined by the following formula (3).

$$S.E.\ \bar{y} = \frac{V_\theta}{4\theta}$$

When both single factors exhibit disturbed segregation, the product method is fully efficient and the variance $\underline{\theta}$ can be obtained from the following formula (3).

$$V_{\theta} = \frac{\theta(2+\theta)(1-\theta)^2}{2(1+2\theta)} \left(\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}\right)$$

The standard error is calculated above.

It has been recognized for many years that the testcross is the most efficient method of mating to detect and determine linkage (15). A homozygous recessive for the genes under study is crossed to an F_1, heterozygote for these genes. A direct determination of the recombinant fraction can then be obtained by taking the number in the recombinant classes as a percentage of the total population (15). Unfortunately it takes two generations to synthesize the recessive backcross parent, and all backcrosses must be hand-pollinated. For these reasons, the F_2 intercross is generally analyzed in linkage studies with higher plants. The efficiency of the F_2 intercross in repulsion and coupling varies as to the intensity of linkage (15). Because of this, it has been suggested that the efficiency for each mating be determined relative to the backcross (3). The following formulae were used to determine the efficiency of the experiment relative to mating (3).

$$\text{Information from backcross} = \frac{n}{y(1-y)}$$

$$\text{Information from } F_2 \text{ repulsion} = \frac{2n(1+2y^2)}{(1-y^2)(2+y^2)}$$

$$\text{Efficiency of } F_2 \text{ repulsion} = \frac{\text{Information from } F_2 \text{ repulsion}}{\text{Information from backcross}}$$

APPENDIX B

Table I. Heterogeneity tests on individual F_2 lots of $\underline{vg}_1$ x $\underline{vg}_2$

	X^2A	X^2B	X^2L	df
Deviation	0.31	0.02	0.52	1
Heterogeneity	1.76	0.87	1.54	2
Total	2.07	0.89	2.06	3

Table II. Heterogeneity tests on individual F_2 lots of $\underline{vg}_3$ x $\underline{vg}_1$.

	X^2A	X^2B	X^2L	df
Deviation	1.98	6.74**	0.66	1
Heterogeneity	1.23	2.25	3.75	3
Total	3.21	8.99	4.41	4

Table III. Heterogeneity tests on individual F_2 lots of $\underline{vg}_1$ x $\underline{vg}_4$.

	X^2A	X^2B	X^2L	df
Deviation	0.71	38.04**	1.29	1
Heterogeneity	5.17	1.91	2.49	5
Total	5.88	39.95	3.78	6

Table IV. Heterogeneity tests on individual F_2 lots of $\underline{vg}_5$ x $\underline{vg}_1$.

	X^2A	X^2B	X^2L	df
Deviation	3.98*	0.42	1.54	1
Heterogeneity	6.83	2.43	0.28	5
Total	10.81	2.85	1.82	6

* indicates a 5% level of significance.
** indicates a 1% level of significance.

Table V. Heterogeneity tests on individual F_2 lots of $\underline{vg}_6$ x $\underline{vg}_1$.

	X^2_A	X^2_B	X^2_L	df
Deviation	0	0.13	2.78	1
Heterogeneity	4.14	12.39	3.08	6
Total	4.14	12.52	5.87	7

Table VI. Heterogeneity tests on individual F_2 lots of $\underline{vg}_3$ x $\underline{vg}_2$.

	X^2_A	X^2_B	X^2_L	df
Deviation	15.03**	0.66	0.34	1
Heterogeneity	4.59	3.11	1.85	5
Total	19.62	3.77	2.19	6

Table VII. Heterogeneity tests on individual F_2 lots of $\underline{vg}_4$ x $\underline{vg}_2$.

	X^2_A	X^2_B	X^2_L	df
Deviation	72.84**	0	3.22	1
Heterogeneity	9.04	10.91	3.21	9
Total	81.88	10.91	6.43	10

Table VIII. Heterogeneity tests on individual F_2 lots of $\underline{vg}_5$ x $\underline{vg}_2$.

	X^2_A	X^2_B	X^2_L	df
Deviation	7.40**	0.06	0	1
Heterogeneity	1.57	3.38	1.03	4
Total	8.97	3.44	1.03	5

Table IX. Heterogeneity tests on individual F_2 lots of $\underline{vg}_6$ x $\underline{vg}_2$.

	X^2A	X^2B	X^2L	df
Deviation	0.23	0.23	0.56	1
Heterogeneity	0.20	4.24	4.33	3
Total	0.43	4.47	4.89	4

Table X. Heterogeneity tests on individual F_2 lots of $\underline{vg}_4$ x $\underline{vg}_3$.

	X^2A	X^2B	X^2L	df
Deviation	95.62**	5.94*	0.08	1
Heterogeneity	1.43	8.77	1.60	8
Total	97.05	14.71	1.68	9

Table XI. Heterogeneity tests on individual F_2 lots of $\underline{vg}_5$ x $\underline{vg}_3$.

	X^2A	X^2B	X^2L	df
Deviation	9.48**	8.89**	0.13	1
Heterogeneity	5.22	4.61	5.19	5
Total	14.70	13.50	5.32	6

Table XII. Heterogeneity tests on individual F_2 lots of $\underline{vg}_6$ x $\underline{vg}_3$.

	X^2A	X^2B	X^2L	df
Deviation	25.53**	2.13	0.44	1
Heterogeneity	6.93	7.91	6.19	6
Total	32.46	10.04	6.63	7

Table XIII. Heterogeneity tests on individual F_2 lots of $\underline{yk}_4 \times \underline{yk}_5$.

	χ^2_A	χ^2_B	χ^2_L	df
Deviation	387.38**	0.42	0	1
Heterogeneity	1.81	9.45	3.47	13
Total	389.19	9.87	3.47	14

Table XIV. Heterogeneity tests on individual F_2 lots of $\underline{yk}_6 \times \underline{yk}_4$.

	χ^2_A	χ^2_B	χ^2_L	df
Deviation	132.16**	51.92**	11.60**	1
Heterogeneity	7.46	7.78	0.75	6
Total	139.62	59.70	10.85	7

Table XV. Heterogeneity tests on individual F_2 lots of $\underline{yk}_6 \times \underline{yk}_5$.

	χ^2_A	χ^2_B	χ^2_L	df
Deviation	3.04	0.87	3.60	1
Heterogeneity	0.53	1.75	1.07	1
Total	3.57	2.62	4.67	2

Table XVI. Heterogeneity tests on reciprocal F_2 lots of $\underline{yk}_4 \times \underline{yk}_3$.

	χ^2_A	χ^2_B	χ^2_L	df
Deviation	95.62**	5.94*	0.08	1
Heterogeneity	0.01	0.03	0.02	1
Total	95.63	5.97	0.10	2

Table XVII. Heterogeneity tests on reciprocal F_2 lots of $\underline{vg}_5$ x $\underline{vg}_3$.

	X^2A	X^2B	X^2L	df
Deviation	9.48**	8.89**	0.13	1
Heterogeneity	0	0	0.14	1
Total	9.48	8.89	0.27	2

Table XVIII. Heterogeneity tests on reciprocal F_2 lots of $\underline{vg}_6$ x $\underline{vg}_3$.

	X^2A	X^2B	X^2L	df
Deviation	25.53**	2.13	0.44	1
Heterogeneity	0.23	2.17	0.67	1
Total	25.76	4.30	1.11	2

Table XIX. Heterogeneity tests on reciprocal F_2 lots of $\underline{vg}_6$ x $\underline{vg}_4$.

	X^2A	X^2B	X^2L	df
Deviation	132.16**	51.92**	11.60**	1
Heterogeneity	0.79	1.80	2.02	1
Total	132.95	53.73	13.62	2

Table XX. Heterogeneity tests on individual F_2 lots of $\underline{neg}_1$ x $\underline{hl}$.

	X^2A	X^2B	X^2L	df
Deviation	4.72*	1.22	83.98**	1
Heterogeneity	1.53	0.49	0.02	1
Total	6.25	1.71	84.00	1

Table XXI. Heterogeneity tests on individual F_2 lots of *neg_1* x *i_1*.

	X^2_A	X^2_B	X^2_L	df
Deviation	4.72*	7.33**	70.44**	1
Heterogeneity	1.53	1.14	0	1
Total	6.25	8.47	70.44	2

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Approved by:

William H. Lachman

Donald N. Maynard

Haroed Rand

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